improved with the use of solvent flattening and averaging over the twofold non crystallographic symmetry and the sequence fit to the resulting electron density. A single data set from selenomethionine HMG-CoA reductase was also collected. Difference Fouriers clearly showed the methionine positions, confirming the chain tracing. The HMG-CoA reductase monomer is composed of two mixed α/β domains. The large domain contains both the amino and carboxy terminal ends, while the small domain is composed of an internal 100 residues. The large domain structure shows a central 24 residue α-helix surrounded on three sides by all α-helical, mixed β/α and β-sheet walls. The small domain is composed of 4 anti-parallel β-strands and two connecting α-helices. Two HMG-CoA reductase monomers form a tightly bound dimer. The core of the dimer is formed by the helical structure of the large domain, including a four helix bundle centered on the dimer axis. Two active site clefts are found at the dimer interfaces with residues from each monomer involved in the formation of the two active sites. Catalytically important residues identified by site-directed mutagenesis line the clefts. Difference Fouriers from native crystals soaked in substrates show that HMG-CoA is bound by the large domain while NADH is bound by the small domain.

PS-03.05.33 The study of Cu(I) and Cu(II) Dopamine β Hydroxylase (DBH) model complexes by X-ray absorption and diffraction

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DBH, a tetrameric glycoprotein of 603 amino acids per monomer, is a copper enzyme which catalyses the transformation of Dopamine into Noradrenaline. According to the hydroxylation reaction mechanism proposed by Klinmann (Brenner, Murray & Klinmann,1986) two copper atoms are involved and play different roles: one is used as electron transfer while the second one, located near the substrate fixation site, directly acts on the dopamine hydroxylation via a CuOOH species. Recently EXAFS studies on the DBH active site brought new insights on the active site by revealing the presence of a S-methionine ligand coordinated to Cu(I), not present in the oxidized Cu(II) form of DBH (Scott, Sullivan, De Wolfe, Dolle, Kruse, 1988; Pettingall, Strange & Blackburn, 1991; Blackburn, 1993).

Several approaches of the study of the DBH structure have been developped in our laboratory. A series of Cu-complexes which are supposed to mimic the structure and the properties of the DBH active site and including N, O and S atoms have been prepared.

X-ray Absorption of nine Cu(I) and Cu(II) model complexes bas been measured on XAS II station at LURE (Orsay-Paris). EXAFS and XANES spectra (Fig. 1) exhibit differences in accordance with the Cu ligation atom nature (N, O, S) and the Cu electronic state.

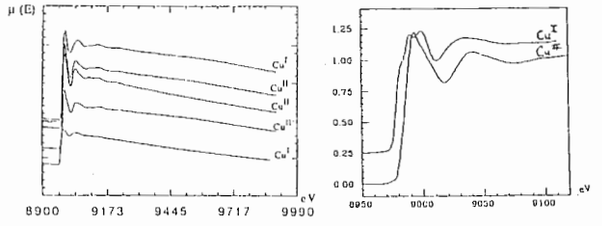

Fig. 1 - EXAFS and XANES experimental spectra of Cu(II) and Cu(I) complexes

The interpretation of our results is in progress and is based on the crystallographic structure obtained for two complexes of this series : an example is given on Fig. 2.

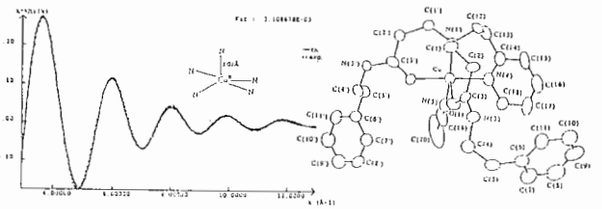

Fig.2 - EXAFS simulation and fit to the experience

The molecular simulation of our models with different force fields (MM2, GROMOS, AMBER) is also used to predict their conformation with some confidentiality with regard to the X-ray diffraction and absorption data.

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PS-03.05.34 CRYSTAL STRUCTURE OF THE MONOFUNCTIONAL CHORISMATE MUTASE FROM *BACILLUS SUBTILIS*

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Chorismate mutase (CMase) is an enzyme in the pathway leading to biosynthesis of aromatic amino acids (shikimate pathway). It is the only characterized example of an enzyme in primary metabolism that catalyzes a pericyclic process, the rearrangement of chorismate to prephenate. So far, the mechanistic details of this enzyme-catalyzed reaction remain a mystery. The simplicity of this rearrangement and the existence of a readily occurring uncatalyzed rearrangement should provide much insight for understanding enzyme-mediated rate-acceleration. Furthermore, catalytic antibodies with chorismate mutase activity have been prepared (Hilvert *et al.*,1988 and Jackson *et al.*,1988) and comparison of the active sites in the enzyme and antibodies will be a novel study to understand catalysis.

CMase from *Bacillus subtilis*, Mr=14.5kD, is the smallest characterized natural chorismate mutase. The size of the enzyme, its Michaelis-Menten kinetics and absence of other associated enzyme activites (Gray *et al.*,1990) result in a simpler experimental system than other bifunctional and more complex chorismate mutases (eg. from *E.coli* and yeast) for structural analysis to understand catalysis. This enzyme crystallizes in space group $P2_1$, with 12 monomers in the asymmetric unit. The crystal structure of CMase was determined using multiple isomorphous replacement method (derivatives - Hg, Ir, Os and a Seleno-methionine protein with 72 Seleniums in the asymmetric unit!). The figure of merit is 0.70 for reflections at 15.0-3.5Å. A polyalanine trace of approximately 76 residues per monomer was made using the MIR map. This constitutes all secondary structure elements and 60% of the asymmetric unit. One round of phase combination using this partial polyalanine model improved the quality of the electron density significantly, especially the side chains and loop regions. The trace of the structure to 2.7Å is almost (87%) complete. Upon completion of the trace, phase extension and refinement will be done to 1.9Å.

Data of complexes with a transition state analog and with the product, prephenate, are also available. Difference fourier maps have revealed the location of the active site. The structural analysis of the active site should provide some long-awaited for mechanistic information on the catalysis by chorismate mutase.

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